ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Forty-sixth Quarterly Report of Progress

Order No. W-13411

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INTRODUCTION

Organisms (spores) of high heat resistance occur naturally in soil. Some species have recently been recovered from soil where space vehicles and components are manufactured and assembled. We have demonstrated from our experiments (39th Quarterly Report) that soil samples obtained from several geographical areas of the United States contained a resistant fraction (about one resistant spore in 1,000). Similar findings have also been reported by other NASA participating laboratories. We have also reported that a hardy organism (CK 4-6) received from the Jet Propulsion Laboratory at Cape Canaveral could be subcultured and sporulated and still maintain its heat resistance.

This report presents a continuation of investigations begun earlier on CK 4-6 spores. The present summary describes the experiments performed on the heat resistant organism CK 4-6 and its response to dry heat at two temperatures (125 C and 135 C) at eight humidity levels ($\leq$ 0.001% to 100% RH) in a closed can system.

I. EXPERIMENTAL

A. Production of spores

Actively growing cells of CK 4-6 were produced by surface inoculating spore growth agar medium. This medium consists of

agar, 3%; Seitz filtered glucose, 0.25%; casamino acids (Technical), 0.25%; yeast extract, 0.5%; $MnSO_4 \cdot H_2O$, 0.001%; and $FeSO_4 \cdot 7H_2O$, 0.0014% in a 250-ml Roux culture flask. The cells were grown for 21 days at 35 C. Spores were harvested by flooding 50 to 70 ml of double-distilled sterile water into the bottle and scraping gently the matted spore surface. The spore suspension was poured in an Erlenmeyer flask containing sterile glass beads and then shaken thoroughly.

After the spores are shaken, the suspension was poured through sterile non-absorbent cotton. Additional double distilled water was passed through the cotton to make a final volume of 150 ml. The spore suspension was centrifuged at 6,000 RPM for 30 min at 5 C. The sediment was resuspended in fresh double-distilled water (100 ml) and placed in a 50-C water bath overnight.

Following this treatment, the suspension was centrifuged and washed five times in 150 ml of double-distilled water at 6,000 RPM for 30 min at 5 C. The final clean spore suspension was observed microscopically (Bartholomew and Mittwers' stain) for debris and percentage of spores present.

The clean spores were stored in double-distilled sterile water at 5 C.

B. Thermal inactivation studies

The spore crop suspension stored at 5 C was sonified and after sonification, dispensed with microburette in 0.01 ml amounts in

stainless steel cups to give about 10^6 spores per cup. The cups were arranged on circular shelves and placed in 200 x 306 tin cans. Each can contained four shelves (30 cups per shelf) for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 110 min at 45 to 50 C (at 1.5-inch Hg pressure absolute).

To increase the drying rate, the oven was purged with dry nitrogen every 10 min for the first 100 min, followed by five consecutive purges of nitrogen, with a vacuum cycle between each purge. After drying, the cans, lids, and contents were removed from the oven, cooled to about 30 C in the equilibration hood, and held overnight in this hood.

An appropriate amount of water was placed in the center cup (B shelf) of each can and sealed immediately. The cans were removed from the equilibration hood, and the seams on each can soldered to prevent leakage of water vapor during the heating cycle.

The cans were heated (125 C or 135 C) for varying times and cooled in a refrigerated water bath. The cans were opened with an electric can opener and sterile microbeads were added in each sample cup (heat treated and nonheat treated), placed in 10 ml of sterile peptone water, and sonified for 24 min. Spore assays were made by the conventional plate count method using trypticase soy agar fortified with 0.1% starch and 0.2% yeast extract. The

plates were incubated for 5 to 7 days at 35 C, and the results were either plotted on semilog paper or the F values were calculated. These F values represent the time to achieve at least a 5-log reduction (i.e., reduce the population from 1×10^6 to < 10).

II. RESULTS AND DISCUSSION

In most cases the inactivation curves at the two temperatures (125 and 135 C) investigated were nonlinear. This is shown in Fig. 1 and 2. In some cases, counts of unheated spores were lower than counts (heated spores) at the early stage of heating. These increases in counts occurred more frequently when moisture (%RH) was higher. It was also observed that after the initial heating period the inactivation curves became linear. As shown in Fig. 1 and 2, it was difficult to see the induction period at relative humidities of 54.7, 68.9, and 100% at 135 C and at 54.7, 73, and 100% RH at 125 C.

Based on F value determinations (Table 1), times to inactivate 10^6 organisms at 125 C were 28, 40, >70, >70, 40, 2, 0.8, and 0.6 h at < 0.001, 0.019, 1.8, 7.3, 29.2, 54.7, 73.0, and 100% RH, respectively. The F values at 135 C were 10, 18, >18, >22, 8, 1.2, 0.6, and 0.5 h for < 0.001, 0.014, 1.38, 6.88, 30.3, 54.7, 68.9, and 100% RH, respectively. The maximum resistance was observed between 1% and 10% RH, with the minimum heat resistance at 100% RH. This organism was observed to be five times more resistant between 1% and 10% RH at 125 C than has been reported for Bacillus subtilis var. niger.

Table 1. Estimates of endpoints at 100% RH or less for experiments at 125 C and 135 C

Temp. C	% RH	Observed time to reach concn./cup, h
125	< 0.001	28
	0.019	40
	1.8	>70
	7.3	>70
	29.2	40
	54.7	2
	73.0	0.8
	100.0	0.6
135	< 0.001	10
	0.014	18
	1.38	>18
	6.88	>22
	30.3	8
	54.7	1.2
	68.9	0.6
	100.0	0.5

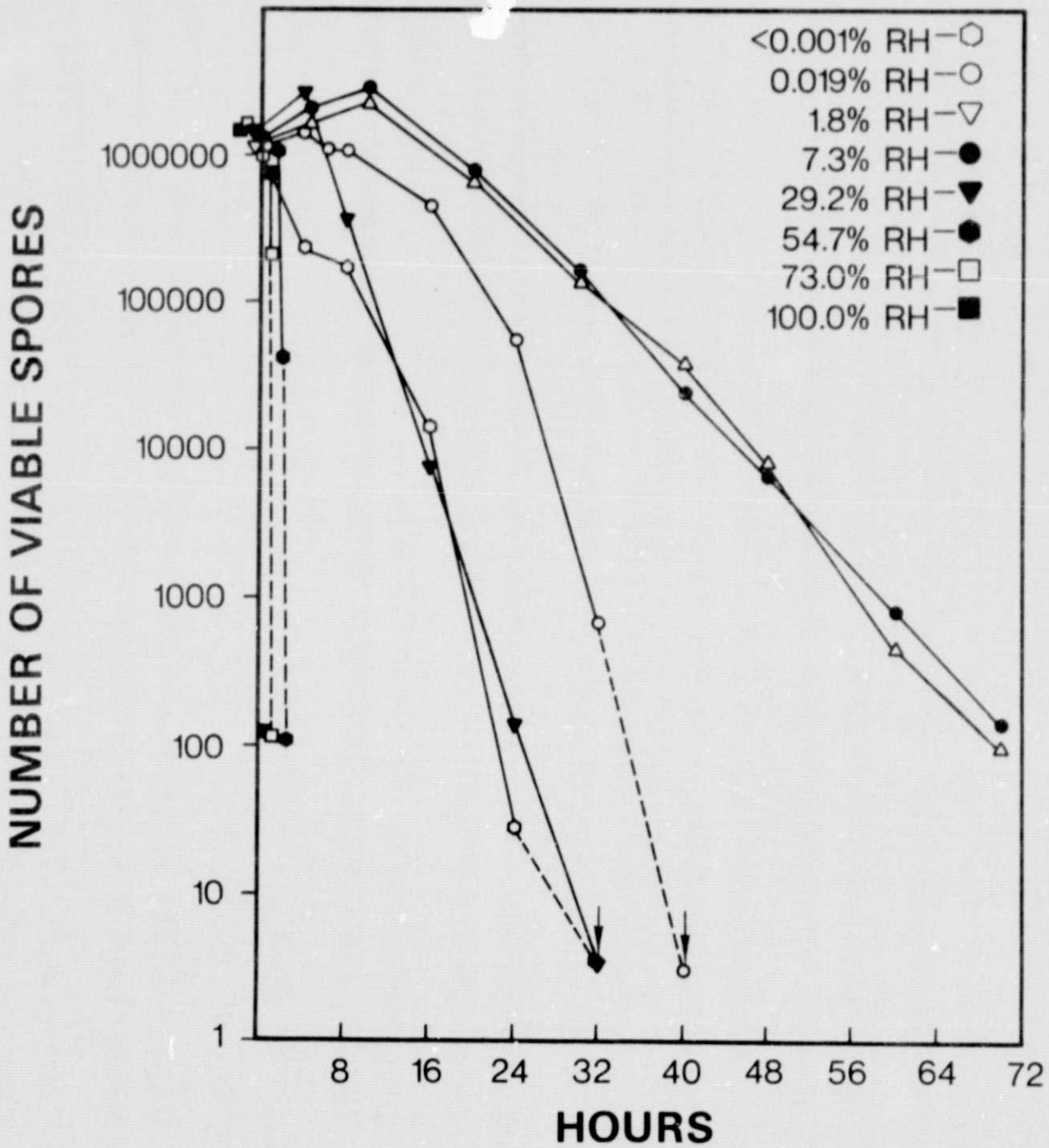

Fig. 1. Dry heat resistance of CK4-6 spores at 125 C and eight relative humidities in a closed can system. (--- = no colonies found.)

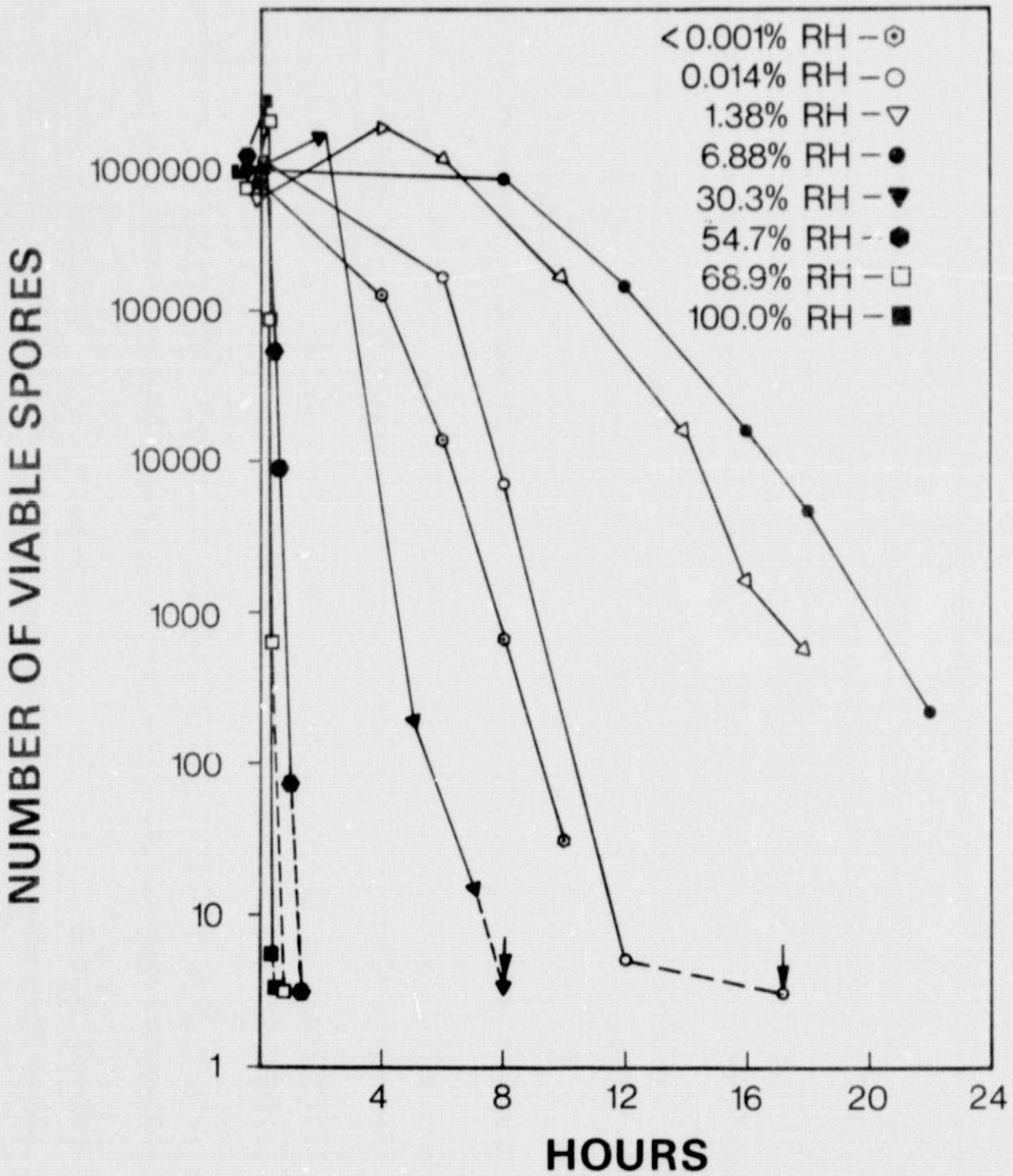

Fig. 2. Dry heat resistance of CK4-6 spores at 135 C and eight relative humidities in a closed can system. (--- = no colonies found.)